Code of Practice for work with Biological Agents and Genetically Modified Micro-Organisms

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1. Introduction

This Code of Practice is a practical guide which lays down the University’s standards for how biological agents [BAs] and genetically modified micro-organisms [GMMs] should be handled, stored, transported and disposed of.

Adoption of the standards outlined in this document will ensure the University fulfils its responsibilities under the Control of Substances Hazardous to Health Regulations 2002 and the Genetically Modified Organisms (Contained Use) Regulations 2003.

Schools and departments where BAs and/or GMMs are used are required to apply these standards and where appropriate use this document to draw up local rules specific to the nature of work and organisms used.

In this code a distinction is intended between the use of ‘must’ which indicates an essential requirement and ‘should’ which leaves room for local discretion following suitable risk assessment.

1.1. Abbreviations

The following abbreviations are used throughout the document.

ACDP: Advisory Committee on Dangerous Pathogens
ACGM: Advisory Committee on Genetic Modification
CL: Containment Level
COSHH: Control of Substances Hazardous to Health Regulation 2002
GMO [CU]: Genetically Modified Organisms [Contained Use] Regulations 2000
GMSC: Genetic Modification Safety Committee
HSE: Health & Safety Executive
BSO: Biological Safety Officer
BA: Biological Agent
GMM: Genetically Modified Micro-organism Organism
PI: Principal Investigator
1.2. Definitions

**Biological Agents.** COSHH defines these as any micro-organisms [virus, bacteria, fungi, yeast etc], cell cultures or human endoparasites which may cause infection, allergy or toxicity or otherwise create a hazard to human health, including any that have been genetically modified. These have been classified into four hazard groups by ACDP on the basis of their ability to infect humans and the consequences of any infection. These classifications are shown below, with some possible examples which may be used within Schools and Departments.

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Unlikely to cause human disease. E.g. Tissues &amp; cell lines of non primate/non human origin. Human/primate cell lines that are well characterised, authenticated, long established and have long history of safe use [e.g. MRC5, HeLa cells] Disabled/attenuated/non-pathogenic strains of some bacteria and virus.</td>
</tr>
<tr>
<td>Group 2</td>
<td>Can cause human disease and may be a hazard to employees; it is unlikely to spread to the community and there is usually effective prophylaxis or treatment available. E.g. tissues and primary cell lines of human/primate origin. Adenovirus, clostridium, most strains of E coli</td>
</tr>
<tr>
<td>Group 3</td>
<td>Can cause severe human disease and may be a serious hazard to employees; it may spread to the community, but there is usually effective prophylaxis or treatment available. HIV, Hepatitis B, E coli 0157, salmonella typhi.</td>
</tr>
<tr>
<td>Group 4</td>
<td>Causes severe human disease and may be a serious hazard to employees; it is likely to spread to the community, and there is no effective prophylaxis or treatment available. E.g. Rabies, Ebola Virus but it is highly unlikely that any such agents would be permitted in the University</td>
</tr>
</tbody>
</table>

This hazard classification is used to determine the appropriate level of containment that must be applied to protect human health. E.g. Group 2 BAs require Containment level 2 [CL2] as a minimum, Group 3 BAs require CL3.

*A genetically modified micro-organism* is a micro-organism [virus/bacteria/yeast/fungi] that has had its genetic material modified in a way that does not occur naturally by mating and/or natural recombination. It may be a BA [human pathogen] and therefore classified under the above system or it may be a plant or animal pathogen, in which case it may pose a threat to the environment. The GMO [CU] Regulations require that containment and control measures must be chosen appropriate to the risk of the activity to both human health and the environment. A list of animal pathogens can be found at on [DEFRA web site](https://www.gov.uk/). Plant pathogens will be dealt with under a separate Code of Practice.
2. Procedures to be adopted before starting work

2.1. Risk Assessments
Any work involving the use and/or propagation of a BA or GMM must have been subject to suitable and sufficient risk assessment. For BAs there is currently no standard assessment form used across the University, nor is there any formal approval process. Therefore schools and departments should ensure that local assessment mechanisms follow the requirement outlined in the COSSH regulations.

For GMMs risk assessments must be completed using the University’s standard GM assessment form which follows the guidance contained in the ACGM Compendium of Guidance. The risk assessment must have been approved by the appropriate local GMSC. Details of this process can be found in the University Policy.

2.2. Notifications
Certain experiments may require notification to the HSE prior to the commencement of the work.

**BAs** – The COSHH Regulations require Group 3 & 4 BAs to be notified to HSE. The introduction of any such organisms must therefore be notified to Safety Office who will arrange for the notification to be made.

Where a School/Department intends to use group 2 organisms it must appoint a local Biological Safety Officer [BSO]. The use of subsequent group 2 organisms must be notified to the local BSO.

**GMMs** – Under The CU Regulations, assessors are required to classify the activity as either Class I or Class II. This classification is directly related to the containment level required for the conduct of the work.

- Containment level 1 = Class 1
- Containment level 2 = Class 2
- Containment level 3 = Class 3

Class 2 activities require notification to HSE and work may commence as soon as HSE has acknowledged receipt of the notification. Class 3 activities will require written consent from HSE. There are fees for these notifications which must be met by the school/department.

Where notification is made under GMOCU, this will satisfy the notification requirements of COSHH.

**The Anti Terrorism Crime and Security Act 2001** requires the University to notify the Home Office of pathogens and toxins listed in Schedule 5 of the Act.

*Provide link – for internal view*

There are no fees or time restrictions for this notification but research workers who wish to work with any organism on the Schedule 5 list should contact the Safety Office.
2.3 **Health Screening & Surveillance.**
Health surveillance and pre–start screening will only be appropriate where an identifiable health effect, related to exposure may occur, there is reasonable likelihood that the effect may occur and there is some means of detecting the effect. This should have been identified by risk assessment and if necessary the worker must be referred to Occupational Health Unit, before commencing work.

If the work involves the use of a Group 3 pathogen or an oncogene records of exposure of individuals must be maintained by the School/Department.

Incidents of unexplained illness should be drawn to the attention of the Occupational Health Nurse by the worker. The Occupational Health Nurse should take appropriate action, consulting the Occupational Health Physician as appropriate.

Work with BAs or GMMs should not preclude staff making blood donations to NBS. However if they work with organisms classified at Group 2 or above they should inform the NBS staff, at the time of donation, of the nature of work they are involved in.

2.4 **Vaccination**
The Occupational Health Unit will provide immunisation for any worker likely to be exposed to any pathogen for which there is effective vaccination. The ACDP Approved List of Biological Agents identifies pathogens for which vaccination is available.

Staff who may as a result of their work be exposed to human tissues or primary human cell lines must receive vaccination against Hepatitis B before starting the work. Staff working with human sputum must be vaccinated against tuberculosis.

It is the responsibility of the Head of School/Departmental to ensure that potential exposure is identified to OHU prior to employment or the work commencing.

*University policy on vaccination of staff working with infectious agents [provide link]*

2.5 **Pregnancy.**
The ACGM Compendium of Guidance states that for most GM work there are no additional hazards in respect of new or expectant mothers. There are however some biological agents that, if infection were to occur, might have more serious consequences during pregnancy or breast-feeding.

Certain BAs within groups 2, 3 and 4 can affect the unborn child if the mother is infected during pregnancy. These may be transmitted across the placenta while the child is in the womb or during or after birth e.g. if the child is breast-fed. Examples of agents that might affect the child in this way are hepatitis B & C, HIV, Herpes, TB, syphilis, chickenpox, brucella and typhoid. For most workers the risk of infection is no higher at work than from living in the community but in certain occupations e.g. biomedical research, nursing, medicine exposure is more likely.

Risk assessments must take account of the nature of the biological agent, how the infection may be spread, how likely contact is and the control measures in place.
These include notification of the hazard, the level of containment for working, protective equipment in use, hygiene measures in place and surveillance measures. The use of available vaccines is recommended, with due regard for contraindications especially in the early stages of pregnancy.

If there is a known or suspected risk of exposure to a highly infectious agent, then it is appropriate for the pregnant worker to avoid exposure altogether.

Rubella (German Measles) and toxoplasmosis can harm the unborn child, as can some other biological agents e.g. cytomegalovirus.

Exposure to these, biological agents should be avoided except where the pregnant woman is protected by her state of immunity.

OHU will perform immunity testing (Hep B, chickenpox, toxoplasmosis, parvovirus, rubella) for risk occupations. Where a pregnant woman proves sero negative, job transfer or temporary leave will need to be considered.

If a worker expects to conceive or believes herself to be pregnant, she may wish to discuss this with the Occupational Health Nurse.

The university policy for New & Expectant Mothers at Work is available on the intranet [Provide updated link](#).

**2.6 Information, Training & Supervision**

Before commencing work, staff involved must have read this COP, any relevant local rules and a copy of the risk assessment. They must have received appropriate training in safe handling of BAs and GMMs and have demonstrated that they are competent. It is good practice to keep records of training; these are mandatory for any staff working at Containment level 3.

Supervision appropriate to the degree of risk and the experience of the worker must be maintained.
3. PRECAUTIONS THAT MUST BE TAKEN WHEN WORKING WITH BAs and GMMs

3.1. General requirements for all work.

- Work may only be carried out in designated containment areas that have been formally approved by the local BSO. Level 3 containment facilities must be approved by the Safety Office. Forms and checklist for the approval and registering of CL 1, 2 & 3 new areas can be found at Appendix I
- Laboratory personnel must receive instructions and training in handling microorganisms, including recombinant micro-organisms and an appropriate standard of supervision of the work must be maintained.
- Eating, chewing, drinking, smoking, storing of food and applying cosmetics must not take place in the area.
- Mouth pipetting is strictly prohibited
- Hands must be washed before leaving the laboratory and immediately if contamination is suspected.
- Lab coats must be worn at all times and removed before washing hands and leaving the laboratory.
- Outdoor clothing and personal belongings such as handbags must not be brought into the laboratory.
- Gloves must be worn if indicated as required by risk assessment.
- Contamination of skin and mucous membranes and self-inoculation must be avoided.
- Sharps should not be used unless essential for the work.
- Procedures must be performed so as to minimise aerosol production.
- Incubators, orbital shakers and fermenters must be located inside designated laboratories.
- BAs/GMMs must be safely stored in designated incubators, refrigerators and freezers. Details of all organisms must be maintained in the department. See Section 11 for more detail on the standard storage requirements.
- Effective disinfectants must be available for immediate use in the event of a spillage
- Waste materials contaminated with BAs/GMMs must be decontaminated before leaving the area, either by suitable disinfection or by autoclaving. See Section 4
- Liquid waste must be suitably decontaminated before discarding to drains.
- Items awaiting disinfection must be stored in a safe manner. Pipettes must be totally immersed in disinfectant after use. Plastic and glassware must be decontaminated as stated in the local code of practice. Guidance on disinfection is contained Section 4.
- Benches and safety cabinets must be cleaned with an approved disinfectant after work is completed.
- Housekeeping must be of a high standard. Avoid storing items such as cardboard boxes underneath benches as these may become contaminated in the event of spillage. This is mandatory in CL2 & 3 facilities.
- All accidents and incidents must be reported to the BSO or deputy.
- Maintenance personnel must always obtain permission from a responsible member of staff in the area involved before entering a restricted area.
- Equipment requiring service or repair which has been in contact with BAs or GMMs must have a signed certificate stating that it does not present any hazards before an engineer is asked to work on it.
3.2. **Containment Level 2 – additional precautions/requirements**

In addition to the above the following must be applied when working at containment level 2.

### 3.2.1. Access

- Experiments must be carried only out in designated Level 2 areas; the door must be closed and must carry a biohazard sign indicating the level of containment.
- Access to the laboratory must be restricted to laboratory personnel and other authorised persons.

### 3.2.2. Handling Procedures

- In general, work may be conducted on the open bench but care must be taken to minimise aerosol production. Where aerosol production is unavoidable a suitable microbiological safety cabinet must be used.
- To prevent spillages within the laboratory, BAs/GMMs or material containing these, must be adequately contained during transfer from safety cabinets and or workstations to other areas of the laboratory, e.g. incubators/orbital shakers.
- Manipulations likely to produce substantial aerosols e.g. shaking, homogenising, sonicating, etc., must be carried out in a Class I cabinet except where the equipment itself is designed to contain the aerosol.
- Procedures such as culturing cells which may produce small amounts of aerosol and require high sterility should be carried out in a Class II cabinet (Specific information on the use of MSCs is included in Appendix II)
- Centrifugation must take place in sealed containers within sealed buckets/rotors. Centrifuges and related equipment should conform to BS EN 60101-0202 1995.
- Glass pipettes &/or sharps must not be used, unless absolutely essential to the work.

### 3.2.3. Use of Bunsen Burners [BBs]

- Exercise due care when using BBs to sterilise loops etc. Any aerosol produced in the flame can be carried in the upward convection current and deposited elsewhere in the lab. Use of plastic loops will remove this risk.
- The convection current produced by BBs will interfere with the airflow of safety cabinets and may compromise operator and product protection. Their use is strongly discouraged. Where a School/Department considers it necessary to use BBs in a cabinet this must be discussed with the University Biological Safety Officer and an ‘in use’ operator protection factor test must be carried out.
- BBs present a fire risk –
  - do not leave on unattended,
  - ensure that they are not located beneath overhanging shelves,
  - ensure flammable liquids and combustible materials such as paper are kept a safe distance away.

### 3.2.4. Waste

- If the autoclave is not within lab, but elsewhere in the building, waste must be transported in robust leak-proof containers.
• Autoclaves used to inactivate waste must be subject to regular validation, calibration and maintenance. See Appendix III for details.
• Where chemical disinfection is used, the disinfection procedure must be validated under working conditions, such as in the presence of buffer solutions or proteins. At containment level 2 it will normally be acceptable to use the disinfectant manufacturer’s data providing that the disinfectant has been shown to be effective against the organism in question and there is strict adherence to the recommended concentration and contact time.
• Disinfection procedures should be included in a standard operation procedure.
• Details of waste disposal are given in Section 4.2

### 3.2.5. Maintenance & Domestic staff.

• In the event of the failure of a piece of plant or equipment requiring attendance by service maintenance personnel, all adjacent surfaces and the equipment must be decontaminated and a signed authority to enter must be obtained from a responsible member of the staff in the area. Details of the University's procedure for entry into lab areas by maintenance staff and contractors can be found at [http://www.nottingham.ac.uk/safety/guides.htm#Entry](http://www.nottingham.ac.uk/safety/guides.htm#Entry)
• Equipment sent for service or requiring repair must be similarly decontaminated and certified safe. There is a form available for this purpose on the Safety Office web site at [http://www.nottingham.ac.uk/safety/forms.htm#decontamination](http://www.nottingham.ac.uk/safety/forms.htm#decontamination)
• Cleaning staff may only enter under the supervision of a responsible member of staff or in accordance with a safe written system of work. This might involve the use of door tags which indicate that the room required cleaning and is safe to enter.

### 3.2.6. Monitoring and testing of control measures.

• Safety cabinets must be serviced & tested in accordance with the requirements of BS EN 12469 2000. They should also be subject to routine maintenance checks at regular intervals. For more detail on the correct use of MSCs see Appendix II
• Autoclaves must be subject to routine maintenance checks and to regular validation and pressure vessel inspections. See Appendix III for detail.
• Centrifuges and their rotors/buckets must be subject to routine cleaning and inspection of seals. Regular service by a competent engineer is recommended for all centrifuges. Servicing and maintenance requirements must be determined following local risk assessment. This should consider the size, operating speed and level of use and the potential damage that could be incurred in the event of a major centrifuge accident, such as rotor disruption. As a rule high speed and ultra centrifuges should be annually maintained and serviced by a competent engineer. Similarly rotors used in large, high speed and ultra centrifuges must be inspected annually by a competent person and records maintained.
• If the risk assessment deems it appropriate monitoring for BAs/GMMs outside of the primary containment may be required. Such methods might include swab testing of surfaces or the use of settle plates.

*Appendix IV* contains model checklists for routine in house monitoring.
3.3. Containment Level 3—additional precautions/requirements

CL 3 laboratory facilities are subject to more stringent management arrangements and will be under the management of a nominated person as detailed in the University’s arrangements for the Design Management & Operation of Containment Level 3 Laboratories [provide link]

3.3.1. Access

- The work must be carried out in a designated containment level 3 laboratory that is separated from other activities and entered via an ‘air lock’. A biohazard sign indicating level of activity must be displayed.
- A suitably qualified and trained individual must be identified as being in charge of the CL 3 facility and will be responsible for its day to day management.
- Access to the room must be strictly controlled and limited to authorised, fully trained personnel. This should be by means of coded key entry system. It may be advisable to maintain a list of authorised people adjacent to the entrance.
- The door to the room must be kept closed when work is in progress and must be locked when room is unoccupied.
- If cultures are being propagated in the room whilst it is unoccupied, the name of the person responsible for the experiment must be displayed on the outer door of the CL 3 facility. This person must be contactable in the event of any emergency.

3.3.2. Handling procedures

- All procedures with infective materials must be carried out in a MSC. Normally a class 1 cabinet will be used to contain aerosol and a Class II cabinet used for tissue culture work where product protection is important. For tissue culture work with organisms that have an air-borne route of transmission a Class III cabinet may be required.
- Centrifuge buckets/rotors must be opened within the MSC.

3.3.3. Growth & Incubation

- Orbital shakers and incubators must be within the CL 3 room.
- Do not use glass flasks in orbital incubators. Plastic conical flasks with 0.2micron filters in the lids are available from laboratory suppliers.
- Flasks must be sealed to prevent release of aerosol into the environment and opened within a MSC. Limit the volume of culture wherever possible to reduce amount that may be released in the event of the flask being dropped/broken.

3.3.4. Equipment

   The laboratory must contain its own equipment as far as reasonably practicable. Equipment should not be removed from the room unless it has been effectively decontaminated. Fumigation may be appropriate in some instances.

3.3.5. Personal Protective Equipment

- Laboratory coats {Howie style} must be worn and should not leave the facility. The use of an alternative colour to the standard white would be appropriate. In
certain area disposable laboratory coats made of suitable material may be deemed appropriate.

- There must be sufficient coat hooks to avoid ‘double hanging.’ Coats must be changed on a regular basis and immediately contamination is suspected. They must be autoclaved before sending for laundering.
- Where risk assessment identifies a requirement, disposable overshoes must be used.
- Gloves [Category 3 PPE tested to ASTM 1571 standard for viral penetration] must be worn at all times and removed before touching items such as telephones and paperwork. The use of two pairs of gloves may be advised, with the outer pair being removed and disposed of within the inner room and the inner pair removed in the air lock before washing hands.
- Where the risk assessment identifies a need respiratory protective equipment may be required to provide additional protection in certain circumstances. E.g. when removing flasks/tubes from orbital incubators and in the event of spillage.

3.3.6. Storage

- Wherever possible cultures should be stored within the CL3 suite.
- It is recognised that in certain circumstances, it may not be practicable to have a −80°C freezer within the CL3 room; however it must be in close proximity to the CL3 suite and must be kept locked once samples have been deposited or moved. There must be robust procedures in place for control of access to any such storage facilities. [See also Section 6]
- Transport between freezer and CL3 suite must be in double containment
- On no account may cultures be grown or propagated outside of the CL3 facility.

3.3.7. Training.

- Staff working at CL3 should have thorough training and have attained a high degree of competence. Training in the first instance should be carried out using less hazardous organisms until competence has been demonstrated.
- Records of training must be kept, and a high standard of supervision must be maintained.

3.3.8. Records of use of Group 3 pathogens by individuals must be kept by the School/Department for 10 years.

3.3.9. Waste

- Waste from CL3 facilities must be inactivated by autoclaving before sending for incineration or being discarded to drains.
- The normal requirement is that the autoclave will be situated within the CL3 lab. If this is not possible the autoclave must be in the adjacent laboratory suite and the waste must be transported in robust leak-proof containers.
- The validation, calibration and maintenance requirements are given in Appendix III.
- Where a chemical disinfectant is used [e.g. for decontaminating surfaces/wiping up small spills etc], the disinfectant must be validated under working conditions, such as in the presence of buffer solutions or proteins.
3.3.10. Maintenance & Domestic staff.

- In the event of the failure of a piece of plant or equipment requiring attendance by service maintenance personnel, all adjacent surfaces and the equipment must be decontaminated and a signed authority to enter must be obtained from the named individual who has responsibility for the CL 3 facility. [http://www.nottingham.ac.uk/safety/guides.htm#Entry]

- Cleaning of the CL 3 facility will normally only be carried out by laboratory staff working in the area. In the event of more specialised cleaning being required this will be arranged and supervised by the named individual who has responsibility for the CL 3 facility.

3.4. Additional precautions when handling recombinant DNA containing potentially oncogenic sequences.

There is no precise definition of an oncogene. ACGM gives advice on what may constitute an oncogene in Part 2B [18 to 23] of the Compendium of Guidance – some examples are given below:

- Viral oncogenes and their cellular homologues.
- DNA sequences which induce tumours in experimental animals
- DNA sequences which cause transformation of cells in vitro e.g.
- Sequences which either cause, or are associated with the “immortalisation” of cells; i.e. sequences which can rescue cells from senescence.
- Sequences whose gene products modulate the expression of growth factors, their receptors or components of the signal transduction mechanisms and lead to escape from normal growth control.
- Sequences which can induce anchorage – independent growth or which can render cells tumourigenic when inoculated into animals.

Control measures to be adopted.
The following procedures set out the additional control requirements required when working with oncogenic sequences as preparations of naked DNA:

- Procedures must be carried out at a designated workstation within a GM area. The risk assessment may indicate that Level 2 is required.
- Gloves must be worn for all work involving oncogenic DNA and must be changed regularly. They must not be worn outside of the designated handling area.
- Sharps must not be used unless essential e.g. scalpel blades may be used for cutting out gel bands and needles for injecting animals.
- Where plastic alternatives exist these must be used in preference to glass.
- All experimental procedures involving naked DNA must be performed so as to minimise aerosol production. Procedures which are likely to generate aerosols e.g. use of blenders, sonicators, homogenisers or vigorous shaking and mixing, etc., must be carried out in a Class I cabinet.
- Certain procedures involving cultured cells may produce small amounts of aerosol but require sterility. These should be carried out in Class II cabinet which has been designated for GM work. Any experiments categorised at Level 2s must be carried out in a designated Level 2 area.
- Centrifugation must be carried out in sealed buckets or rotors and should be transported to the centrifuge already sealed. Buckets/rotors must be opened within an MSC.
- Decontamination of naked DNA should be carried out using a high pH detergent e.g. DECON 90 at 5% concentration. This solution should also be available for swabbing surfaces in the event of spillages.

**Records of Exposure**

Schools/Departments must ensure that there is a recording system in place that enables individuals who have been involved in work with oncogenes to be identified. This is achieved by ensuring that all workers involved in the project are named on GM risk assessment and that this is reviewed and updated accordingly.

Where an individual is potentially exposed to an oncogene [spillage/sharps injury, this must be recorded on the University Accident/Incident reporting system. The Safety Office must be immediately informed as such an occurrence may be reportable to the HSE.

The individual should be referred to Occupational Health who will ensure an appropriate entry is made on the individual’s health record.

The above records must be kept for 40 years and be available for inspection.

**3.5. Work with material/tissues of human/primate origin.**

The hazards associated with blood, tissues and body fluids from human donors are equivalent to Group 2 biological agent, unless the donor is known or suspected to be suffering from a human pathogen that falls into a higher group. E.g. HIV, Hep B.

Where research work within the University involves the use of human tissues, the following policy must be adopted:

- All potentially exposed staff must receive vaccination against Hepatitis B.
- Wherever possible the blood or tissue must be obtained from screened sources. e.g. National Blood Service
- Collaborating clinicians supplying blood or body tissue must be informed in writing by the Head of School that they must take steps to ensure that they do not provide material from any of the following groups.
- Patients who have been identified as HIV, Hep B positive or from high risk groups such as intravenous drug users etc.
- Patients who are/may be suffering from any life threatening transmissible human disease,
- Samples must be clearly labelled and identifiable in the event of the material being involved in any exposure incident. The name of donor, clinician supplying the samples and where appropriate the hospital number of the donor should be recorded. In the case of ‘blind trails’, there must be some means of obtaining this information.
- Where samples are to be imported from overseas then consideration must be given in the risk assessment to any endemic diseases or parasitic infections that may be endemic in the country of origin. E.g. HIV in certain African states, and to samples which may contain parasites.
• All research involving the use of human body parts, including blood and serum must have received ethical approval.

3.5.1. Blood donations by staff
Where research work within the University necessitates the use of staff blood donations the following standards must be met.

• Phlebotomy may only be performed by a competent person. The Head of School must have procedures in place to ensure the competency of individuals can be established and have a system of authorisation.
• All donations of blood, by staff/students for research purposes must be voluntary, unpaid and without coercion and signed consent obtained.
• Persons from HIV or Hep B high-risk groups, or who consider themselves to be at risk, must not donate blood for research purposes.
• Blood must not be taken in laboratories, offices or general workrooms. A special room must be set aside for this purpose, or if this is not feasible arrangements must made to have this done in a nearby clinical area.
• Blood donated by staff must not be used to set up primary cultures [see below]

Phlebotomists must follow the following rules.

• Wear a clean lab coat or gown [not the one worn in the laboratory for their work.]
• Wash hands between donors – cuts and grazes must be covered.
• Ensure needle used is the correct gauge and is sterile.
• Remove needle from syringe before discharging contents. Do not re-sheath the needle; discard it directly into an approved sharps container.
• Needles must be removed directly into a sharps bin using the safe removal device in the lid of the bin, forceps or similar device to distance hands from the needle.
• After removing needle, transfer blood carefully into specimen container, so as to avoid production of aerosol and contamination the outside of the container.
• Label container clearly with name of donor and date of donation.
• At the end of the procedure swab the area and arm rest with a suitable disinfectant.
• Transport specimens to lab areas in robust secondary containers to avoid spills.

Records
Each blood donation should be recorded with the date, place name of donor, amount of blood taken and name of phlebotomist. The donor must also be asked to sign against the record to indicate their consent.

Ethical issues
Consideration needs to be given to the possible outcomes of working with staff donations. For example if the research work revealed that a colleague had a rare chromosome abnormality, or an abnormal growth factor production this could lead to confidentiality and ethical issues. For these reasons the Head of School must be informed before any such work is undertaken so that consideration can be given to whether this work is necessary and or appropriate.
3.6. **Work with cell cultures**

Cell cultures may cause harm to human health and are therefore included in the definition of a ‘biological agent’.

The hazard and risk to human health will vary depending on the nature of the cells being handled. E.g. Cells of non primate animal origin, well established and characterised human cell line present a minimal risk to the worker and can be handled at level 1. Cell lines obtained from The National Institute of Health or the American Type Culture Collection are characterised and can normally be handled at containment level 1, unless accompanying information states otherwise. Unscreened cells, cells with a less well-defined history and primary cultures from ‘normal’ human donor must be handled at containment level 2.

Table 1 on the following page has been compiled from guidance issued by HSE in the ACGM Compendium in respect of selection of an appropriate containment levels.

In addition to the controls identified earlier in this section the following additional precautions and practices should be observed in relation to cell cultures.

- Only cell strains that have been authenticated and/or have documented history of safe use should be used.
- Avoid cross contamination of cultures by handling only a single cell line at any one time, with appropriate decontamination procedures between operations.
- All procedures in the MSC should be carried out so as to minimise aerosol production.
- Be aware of culture medium contains components [e.g. serum or antibiotics] that have sensitising properties.
- Where cells are deliberately infected with a virus or other organism the appropriate level of containment must be applied for the particular agent.
### TABLE 1

<table>
<thead>
<tr>
<th>Type of cell line</th>
<th>Containment requirement</th>
</tr>
</thead>
</table>
| Non human, non-primate cell lines including those of plant, insect and human origin. | Containment level 1, plus apply principles of good microbiological practice and good occupational safety and hygiene.  
[A Class II MSC is likely to be used purely to provide product protection, not for reasons of safety] |
| Well characterised or authenticated finite or continuous cell lines of human or primate origin that have a low risk of endogenous infection with a human pathogen, presenting no apparent hazard to the laboratory worker or the environment |                                                                                       |
| Finite or continuous cell lines/strains of human or primate origin that have not been fully authenticated or characterised, except where there is a high risk of endogenous pathogens such as blood born viruses. | Containment level 2 plus work in a Class II MSC [provides both product and operator protection]. |
| Primary cells from blood, lymphoid cells, neural tissue of human or simian origin. | Containment to be appropriate to the potential risk. For material ‘normal human’ donors this will usually be Containment level II unless the source of the tissue is questionable. |
| Cell lines with endogenous pathogens or cells that have been deliberately infected with a harmful biological agent. | Containment to be appropriate to the biological agent. |

#### 3.6.1. Primary cultures

A major concern with primary cultures is the possibility that they may undergo spontaneous transformation. This tends to happen more frequently in rodent cells, but can occur with human and primate cells.

It is important that staff are aware that transformation of cells isolated from self or a close colleague in the same area can pose a hazard to that individual. If re-introduced back in the circulatory system of the host it will not be recognised as foreign by the immune system.

If there is suspicion that a natural transformation of a primary culture has occurred or at any stage there is knowledge or suspicion that a cell culture contains a harmful biological agent, the work must be re-assessed and if necessary transferred to a higher level of containment.
The following signs that may indicate transformation or presence of a virus particles:

- Changes to phenotype of cell, e.g. alteration of shape, size, and cell confluence, loss of contact inhibition.
- Speed of cell division

**Additional precautions for work with primary cultures:**

- Never culture or deliberately transform you own cells or those from immediate colleagues.
- Wherever practicable culture primary cells for a short term period. In case of blood cultures this will normally be for 48-72 hours maximum. This will reduce the possibility of spontaneous transformation to virtually zero.
- Where possible use tissues from screened sources and/or low risk sources.

### 3.6.2. Genetically Modified Cell Lines

Where cell lines are to be infected/transfected with a GMM it will be necessary to consider the risks under both the GMCU and COSHH regulations.

The assessment under COSHH should consider the hazardous properties in relation to the safety of the worker. The GM risk assessment will be concerned with the properties of the cell line when modified by the GMM and will determine the containment and class for that GMM. It may be that when the risk assessments are completed the containment levels are different. In such cases the higher level of containment must be adopted.

For example the COSHH assessment may require the cells to be handled at CL 2 but the GM assessment identifies that the actual GMM can be assigned to CL 1 as there is no additional hazard conferred. In this case the cells must be handled at CL 2 but this will not necessitate notification of a Class 2 activity under GMCU Regulations.

### 3.7. Experimental work with transmissible spongiform encephalopathy agents [TSEs]

#### 3.7.1. Introduction

The term TSE describes a rare and fatal degenerative condition of the central nervous system occurring in man and certain animal species which is transmissible.

The **human** TSEs are:

- Creutzfeldt Jakob disease (CJD) including classical sporadic; familial; iatrogenic and new variant;
- Gerstmann Sträussler Scheinker syndrome (GSS);
- fatal familial insomnia (FFI);
- kuru.

The **animal** TSEs are:
- scrapie in sheep, goats and moufflon;
- bovine spongiform encephalopathy (BSE) in cattle;
- transmissible mink encephalopathy (TME) in farmed mink;
- chronic wasting disease (CWD) in deer species;
- feline spongiform encephalopathy (FSE) in domestic cat and captive exotic felines;
- spongiform encephalopathy in captive exotic ungulates.

3.7.2. Properties of the agents

All TSEs exhibit unusual resistance to conventional decontamination methods normally used in laboratories. They are resistant to standard chemical agents such as formalin and are very resilient to high temperatures.

3.7.3. Modes of transmission

TSEs have been shown to be transmissible experimentally to animals by inoculation. There have been no confirmed cases of any occupationally acquired infection of any TSE. The most likely route of occupational exposure would be from infected tissues or materials by direct inoculation (e.g. puncture wounds, 'sharps' injuries or contamination of broken skin), by splashing of the mucous membranes or, exceptionally, by swallowing.

3.7.4. Tissue distribution.

The following table shows the distribution of TSE in tissues and body fluids.

<table>
<thead>
<tr>
<th>High risk tissues</th>
<th>Medium risk tissues</th>
<th>Low risk tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>Major peripheral nerves</td>
<td>Milk</td>
</tr>
<tr>
<td>Spleen</td>
<td>Cerebro-spinal fluid</td>
<td>Semen</td>
</tr>
<tr>
<td>Tonsil</td>
<td>adrenal gland</td>
<td>Urine</td>
</tr>
<tr>
<td>Eye</td>
<td>Lung</td>
<td>Blood</td>
</tr>
<tr>
<td>Lymphoid tissue</td>
<td>Pancreas</td>
<td>Saliva</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>Liver</td>
<td>Skin</td>
</tr>
<tr>
<td>Pituitary gland</td>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>Dura mater</td>
<td></td>
<td>kidney</td>
</tr>
<tr>
<td>Thymus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placenta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral lymph nodes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.7.5. Hazard categorisation

The human TSE agents are classified in Hazard Group 3 because of the severity of infection. However, as it is recognised that the TSEs are unlikely to be transmitted by the aerosol route, derogation from full Containment Level 3 is allowed. This means that, subject to local risk assessment, certain containment measures may be dispensed with.
BSE, and diseases in other species caused by the BSE agent (spongiform encephalopathy in felines and in captive exotic ungulates), are invariably fatal, once clinical signs have appeared in the animals they affect. A link between BSE in animals and nvCJD in man has also been established, although there remain uncertainties about, for example, possible routes of exposure. BSE and related agents are now classified in the same Hazard Group as the agent responsible for CJD, i.e. Hazard Group 3 with derogation.

Other animal TSEs (e.g. scrapie) are invariably fatal in the animals they infect. In view of the novel features of all TSE agents, and the uncertainty about infectivity in man, it is recommended that prudent precautions are taken when working with tissues or preparations from infected animals. However, as there is no evidence of transmission of scrapie to humans it is not formally classified as a biological agent or placed in a Hazard Group.

However a precautionary approach should be adopted in respect of scrapie and animal TSEs as shown in the table below.

### 3.7.6. Containment Levels recommended for experimental work with the agents of TSEs

<table>
<thead>
<tr>
<th>Work with:</th>
<th>Lab containment level</th>
<th>Animal containment level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human TSE agents</td>
<td>3 [derogation may be applied subject to risk assessment]</td>
<td>3 – for small animal work ** [derogation may be applied subject to risk assessment]</td>
</tr>
<tr>
<td>BSE and other related animal TSE agents (FSE, SE in captive exotic ungulates TME and CWD)</td>
<td>1 for large animal work</td>
<td></td>
</tr>
<tr>
<td>Scrapie</td>
<td>2</td>
<td>2 for small animal work **</td>
</tr>
<tr>
<td>**</td>
<td>1 for large animal work</td>
<td></td>
</tr>
</tbody>
</table>

** Risk of exposure is considered greater from work with small animals because of potential for biting and scratching. Larger intact animals present a remote risk and therefore lower containment level is appropriate.

For certain work derogation from full CL 3 may be allowed subject to nature of work and local risk assessment. This is because the most likely route of infection is by the percutaneous route and to a lesser extent by ingestion. The following derogations may be considered in the risk assessment process.
Negative air pressure
Subject to local risk assessment, it may not be necessary to maintain the laboratory at an air pressure negative to atmosphere, as would normally be required for work at Containment Level 3. However, if the laboratory is mechanically ventilated, it must be maintained at an air pressure negative to atmosphere while work is in progress. In practice, when in use, the airflow through a ducted microbiological safety cabinet will usually mean that the laboratory is maintained at negative pressure.

Sealability
The TSE agents are largely unaffected by normal fumigants, therefore, for the purposes of TSE containment, there is little practical benefit to be gained by making the laboratory sealable for fumigation. However, the local assessment should consider what decontamination methods would be used, particularly in the event of a major spillage.

HEPA filtration
Where a laboratory is mechanically ventilated, it may not be necessary for all extract air to be HEPA filtered, subject to local risk assessment. (Any air exhausted through a microbiological safety cabinet would in any case be HEPA filtered).

3.7.7. General basic precautions for experimental laboratory work with human or animal TSE agents or high / medium risk tissues from known/suspected infected sources.

The following general precautions should be adopted to minimise risks:

- Dedicated room with access restricted to authorised individuals
- Eye protection at all times.
- Gloves at all times
- Use MSC if aerosols could be produced.[ e.g. when homogenising potentially infective tissues]
- Use ‘single-use’ disposable items and/or dedicated equipment wherever possible.
- Avoid/minimise use of sharps. Where use is essential [e.g. post mortem examinations, cutting infected tissues] consider the use of armoured [Kevlar] gloves.
- Work over tray with disposable plastic liners that can be removed and incinerated to avoid need to decontaminate surfaces.

3.7.8. Post mortem [PM] examination of animals with TSE

The first consideration must be whether it is necessary to do a post mortem or whether such exposure can be prevented and the appropriate containment level and controls for the procedure. See table above.

If it is necessary to undertake a large animal PM the Safety Office must be informed and will contact the HSE for advice.

The following basic precautions should be implemented for small animal PMs.

- ensure that the Containment Level of the post mortem area is appropriate for the agent involved. Where it is not possible to use a dedicated room, an area of the post mortem room should be set aside;
• consideration should be given to the subsequent disinfection of working surfaces, for example, work may be conducted in a stainless steel or enamel tray which can then be autoclaved. Other working surfaces should be protected by disposable coverings;
• the procedure should be planned so that all equipment required is readily to hand and work should be organised so that there are no interruptions (e.g. to answer the telephone);
• ‘single-use’ disposable items should be used wherever practicable; alternatively a set(s) of dedicated instruments may be used;
• protective clothing including gloves, gowns, masks and visors or safety spectacles should be worn;
• a ‘clean’ assistant should be available to take care of record-keeping, and handing over instruments etc;
• procedures for disinfection and decontamination as described in below should be followed.

Pat 2A Annex III of the ACGM Compendium of Guidance gives detailed information on the issues to be considered when carrying out GM work with PrP genes. This should be referred to when carrying out the risk assessment. The following table summarises the recommended containment levels for different types of GM work with PrPs

<table>
<thead>
<tr>
<th>Work involving</th>
<th>Containment level for work with pro and eukaryotic GMMs</th>
<th>Containment level for work with transgenic animals expressing PRP transgenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expression of normal human or animal PrP genes</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[small or large animal work]</td>
</tr>
<tr>
<td>Expression of modified human/animal PrP gene where there is no expectation that the modified protein will have the ability to convert human PrP to pathogenic isoforms</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[small or large animal work]</td>
</tr>
<tr>
<td>Expression of any modified human/animal PrP gene known or intended to have the ability to convert human / animal PrP known or intended to</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>small animal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>large animal</td>
</tr>
</tbody>
</table>
Non expression activities in disabled hosts [micro organisms] where the gene codes for any modified human or anima PrP known or intended to have the ability to convert human PrP to pathogenic isoforms

| 2 | N/a |

Non expression activities in disabled hosts [micro organisms] where the cloned gene codes for normal human or animal PrP genes or modified human/animal PrPgene where there is no expectation that the modified protein will have the ability to convert human PrP to pathogenic isoforms

| 2 | N/a |

**Note:** Where CL 3 is indicated derogation from full CL 3 requirements in respect of negative pressure, sealablity and HEPA filtration may be applied subject to risk assessment.

### 3.7.10. Decontamination and disposal of waste

The agent thought to be responsible for CJD is recognised as being particularly resistant to standard physical and chemical methods of inactivation and decontamination. The following regimes can be used:

- Autoclaving in porous load [high vacuum] autoclave at 134°C for minimum of 18mins followed by incineration. Downward/upward displacement autoclave must not be used.
- Where autoclaving is not practicable treat with hypochlorite 20,000ppm for 1 hour.
- Where surfaces have to be decontaminated use hypochlorite 20,000ppm. Constant re-wetting of the surface will be required over the treatment period of 1 hour. For this reason careful consideration must be given to the choice of work surfaces. Alternatively 2M sodium hydroxide can also be used but this is not as effective as hypochlorite and again constant re-wetting of the surface is required.

#### i) Instruments

Where the use of none disposable items cannot be avoided, these must first be cleaned at least twice before autoclaving or chemical disinfection [see above]. First treatment should be in a dedicated ultrasonic bath and the second in a dedicated automated thermal washer/disinfector using neutral or enzymatic detergents.

#### ii) Histology and inactivation of samples.

Tissues for histological examination should be immersed in 96% formic acid for 1 hour after routing fixation. If tissues have been exposed to phenol during processing do not expose them to formic acid. These must then be treated as infective tissues.

Paraffin blocks and sections not previously treated with formic acid should be immersed in 95% formic acid for 5 minutes after de-waxing.
Solid tissues should be autoclaved and then incinerated. Body fluids should be autoclaved or treated with 20,000ppm hypochlorite before incineration.

**iii) Animal carcasses**, and other waste from animals experimentally infected must be incinerated. Bedding and faeces from larger animals must be incinerated where there is a risk of shedding infectivity.

**iv) Spillages**
Minor spillage should be wiped up with tissue soaked in disinfectant and surface treated as outlined above.
Large spillage. Wear gloves and apron over lab coat. Soak up using absorbent material and disinfect surfaces as above. Absorbent material, gloves and apron should then be double bagged and sent for incineration.

**v) Decontamination of Microbiological Safety Cabinets [MSCs]**
Formaldehyde, the normal fumigant for safety cabinets is not effective against TSE agents.

MSCs used for work with TSE should be of the type that has the facility for safe change of HEPA filters by bagging. In addition the surface the HEPA must be sprayed with hair spray or similar in order to limit shedding of particulate matter.

For class II cabinets the main HEPA should be located beneath the work surface to prevent contamination of the plenum.

Treat pre-filters with 2M NaOH to limit dust dispersal and contain securely for incineration.

### 3.7.11. Records of exposure
Under certain circumstances COSHH requires employers to keep a list of employees who are exposed to HG3 or 4 agents. The decision to keep a list depends on the local risk assessment. For TSE agents a list is only required where employees deliberately work with the agent. For example:

- those involved in laboratory research work and veterinary clinical work with a TSE agent;
- laboratory staff handling tissue specimens from patients with CJD of any type, in either routine or specialist neuropathology laboratories
- staff undertaking post-mortem examinations of patients who have died of CJD of any type or where CJD of any type is suspected.

### 3.7.12. Reporting Accidents.
Any accident must be reported to the Safety office in accordance with normal University procedures. However the Reporting of Injuries, Diseases and Dangerous Occurrences Regulations (RIDDOR)1995. requires some accidents and exposures to be notified to HSE. These include any infection reliably attributable to work with live or dead humans or animals, exposure to blood or body fluids or any potentially infected material derived from any of the above. Accidents or incidents, which result in or could result in the release or escape of a TSE agent, must also be reported under RIDDOR as a dangerous occurrence. This would include a needlestick or sharps injury involving a sample known to be infected with a TSE.
3.7.13. **Health Surveillance**

There are no valid techniques for detecting early indications of TSE disease at the present time. Health surveillance will therefore be limited to setting up and maintaining individual health records for employees likely to be exposed to TSE agents.

4. **Waste disposal procedures**

The following information lays down the general principles and standards for treatment of waste. School / departmental laboratory codes should reflect local circumstances whilst ensuring that the standards and principles detailed below are met.

All waste must be inactivated by validated means before pouring down drains or leaving the site for incineration. Inactivation may be achieved by autoclaving or disinfection.

4.1. **Autoclaving**

- This is the preferred means of inactivation and is mandatory for all waste generated in CL3 facilities. In other areas it must be used for waste that cannot be effectively inactivated by disinfection.
- Such items might include: Agar plates, cell/bacterial pellets, small tubes, plastic loops, pastettes, tips and gloves.
- Portable, benchtop laboratory autoclaves are **not** suitable for the inactivation of waste as they do not have the necessary cycle features for waste inactivation. If in doubt consult the Safety Office.
- Cycle times and temperatures will need to be determined for each autoclave and type of load. As a guide 121°C for 15 minutes is an absolute minimum. This temperature must be achieved at the centre of the load and timing must begin when the entire load reaches the required temperature.
- Further information on the principles of effective steam sterilisation, safe operation and necessary maintenance and validation checks for autoclaves can be found in **Appendix III**

4.1.1. **Bags & Containers**

- Proprietary autoclave bags should be used. These should not be filled more than ¾ full in order to allow the necks to be closed.
- Some means of identifying the source of the waste must be shown [school/department/lab number]. This will depend on local policy but might include coded ties/tags or origin indicator tape.
- Bags should be transported to the autoclave in robust leak-proof containers.
- On removal from the autoclave the waste should be placed in a yellow clinical waste bag and sent for incineration. The source of waste must again be identified.
4.2. Disinfection

- This generally refers to the use of chemical agents to destroy the potential infectivity of a material. Effective disinfection is dependent upon:
  - activity,
  - concentration and
  - length of contact.

- Disinfection is not as effective as steam sterilisation in destroying viable organisms, nor is it easily monitored.
- Disinfection must not be used for treating waste from CL 3 facilities.
- Disinfection is a suitable means of inactivation for:
  - Reusable items that are heat sensitive
  - Liquid wastes and effluents other than cultures containing Group 2/3 BAs/GMMs
  - Liquid cultures of, and equipment used in association with, Group 1 organisms.
  - At the end of the work for decontamination of surfaces and equipment that cannot be autoclaved.

4.2.1. Choice of disinfectant

- Before choosing a disinfectant it is important to refer to the manufacturers’ data to ensure that the disinfectant will be effective against the organism(s) in question and to determine the recommended concentrations and contact times. Bear in mind when choosing a disinfectant that the aim is to reduce the titre by at least 5 logs and that large amounts of protein can interfere with chemical inactivation.
- Local School/Departmental codes of practice must identify the disinfectant used within the area, its use dilution(s), how often it should be changed, the contact time to ensure inactivation.
- Appendix V gives some general guidance on disinfectants

4.2.2. Validation of Disinfectants

For group 1 and 2 BAs/GMMs it will normally be sufficient to rely on the manufacturer’s data, providing the recommended concentrations and contact times are used. For Group 3 BAs/GMMs, departments must demonstrate the effectiveness of the disinfectant with the relevant organism under the specific conditions of use.

4.3. Disposal of Equipment

- Any equipment that has been used in association with the growth of BAs/GMMs must be decontaminated by suitable means before it is sent for disposal. A certificate of decontamination must be completed – available of Safety Office website at http://www.nottingham.ac.uk/safety/forms.htm#decontamination
- For most items surface decontamination with suitable disinfectant will be acceptable, however the following items will require fumigation prior to disposal:
  - Microbiological safety cabinets.
• Equipment used in association with hazardous BAs/GMMs that cannot be effectively disinfected.
• **Fumigation with formaldehyde is an extremely dangerous operation and must be subject to thorough risk assessment and a safe operating procedure written. Only trained and competent person may carry out this procedure. Guidance on fumigation is contained in Appendix V**

5. **Action to take in the event of spillage/accidental release of BAs/GMMs**

When drawing up contingency plans a number of different factors/scenarios will need to be considered to determine the most appropriate course of action:

• **Type of agent** - the Hazard Group, route of transmission, infectious dose (if known), stability in the environment.
• **Type of accident** - instantaneous or delayed - for example, a dropped flask as compared to a broken centrifuge tube which may be undiscovered until centrifuge is opened.
• **Severity of accident** - amount and concentration of material that could potentially be released and its form, for example, is aerosol formation likely?
• **Numbers of staff potentially exposed** - this may depend on location of accident (see below).
• **Location within the laboratory** - an accident in the open laboratory may require evacuation, as compared to a more 'contained' accident in a microbiological safety cabinet.
• **Room air change rate** - this needs to be known to enable an assessment to be made of the time needed before staff can safely re-enter the laboratory after a spillage.
• For any accident involving broken skin, bleeding should be encouraged and the procedure for skin contamination adopted. A First Aider should be called.
• Contaminated clothing must be removed as quickly as possible to avoid infective materials reaching the skin, placed in autoclave bags and autoclaved. If the skin is contaminated wipe area with disinfectant and then wash with antiseptic soap and water.

5.1. **General**

• In the event of significant spillage inside the laboratory immediate evacuation may be required. This will depend upon the nature of the BA/GMM and should be identified in the risk assessment.
• Spillage procedures, relevant to the type of organisms in use in the area, must be prominently displayed.
• Remove any contaminated clothing – leave in lab or lobby.
• Leave MSC running or switch on before leaving lab.
• Secure door and post signs to prevent others entering.
• Decontaminate skin with disinfectant. If necessary and available use emergency shower.
• Depending on nature and duration of exposure and agent of release seek immediate medical assistance if warranted. [This should have been identified at the stage of risk assessment]
5.2. **Spillage in CL 3 facility.**

- Regardless of whether the agent presents a risk of aerosol transmission the room must be evacuated and cleared of infectious aerosol and then fumigated.
- Assessing the time for clearing aerosol depends on:
  - Concentration of organism in solution spilled.
  - Quantity spilled
  - Room ventilation air change rate.

The following table can be used to calculate the airborne concentration for a given volume of culture at a given concentration.

**Table 2** Airborne concentration of micro-organism/m$^3$ vs. volume and initial solution concentration.

<table>
<thead>
<tr>
<th>Soln. Conc. /ml</th>
<th>Small &lt;50ml</th>
<th>Med. 50 to 500ml</th>
<th>Large &gt; 500ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{10}$</td>
<td>5X10$^6$</td>
<td>5X10$^7$</td>
<td>5X10$^8$</td>
</tr>
<tr>
<td>$10^9$</td>
<td>5X10$^5$</td>
<td>5X10$^6$</td>
<td>5X10$^7$</td>
</tr>
<tr>
<td>$10^8$</td>
<td>5X10$^4$</td>
<td>5X10$^5$</td>
<td>5X10$^6$</td>
</tr>
<tr>
<td>$10^7$</td>
<td>5000</td>
<td>5X10$^4$</td>
<td>5X10$^5$</td>
</tr>
<tr>
<td>$10^6$</td>
<td>500</td>
<td>5000</td>
<td>5X10$^4$</td>
</tr>
</tbody>
</table>

This assumes a worse case scenario where aerosol potential is high but exposure time is short due to immediate evacuation. The aerosol potential is a measure of how much of suspension spilled becomes aerosolised.

- Before staff re-enter the lab sufficient time must be allowed for any aerosol to be removed from the room and fumigation to be carried out. **Table 3** below indicates the number of minutes for a given number of air changes required to remove 90, 99 or 99.99% of airborne contaminants.
### Table 3

<table>
<thead>
<tr>
<th>Air changes per hour</th>
<th>Percentage % Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>40</td>
<td>3</td>
</tr>
</tbody>
</table>

**Example spillage**

A flask containing $20 \text{ ml}$ of a $10^8$ spores/ml suspension of Bacillus anthracis is accidentally dropped on the laboratory floor. The laboratory ventilation rate is 12 air changes per hour.

From Table 2, the airborne concentration is 50 000 spores/m$^3$ on leaving the laboratory. From Table 3, after 58 minutes, 99.99% of the airborne spores will have been removed, leaving a concentration of 50 spores/m$^3$.

After a further 35 minutes, a further 99.9% of the remaining spores will have been removed, and the concentration will have dropped to 0.05 spores/m$^3$, i.e. the laboratory will be almost free of any airborne spores.

When the laboratory has been rendered safe to re-enter the spill should be contained using absorbent booms and appropriate disinfectant applied [e.g. Virkon powder or Trigene at 10 %] Absorb on paper towels and/or scrape into an autoclave bag. Autoclave prior to disposal.

- Care must be taken with broken glassware. Do not pick up by hand, use tongs/forceps and dustpan and brush [autoclave after use].
- Extensive cleaning of the floor is likely to be required where a flask has been dropped, as contents will have contaminated areas far away from point of impact.
For this reason floors under benches in CL 2 & 3 laboratories should not be cluttered with boxes or other absorbent items.

5.3. Significant spillage in CL2 facility.

- In the event of an accident resulting in significant spillage inside the laboratory the safety cabinet should be left to run until the room is cleared of infectious aerosol (see Tables 1&2).
- Staff, who have been properly trained, can then re-enter the laboratory wearing appropriate personal/respiratory protective equipment.
- The spillage should then be dealt with as previously described, again assessing the nature of the spillage in determining the extent of cleaning required.
- Spillages of CL2 agents which do not present a risk of aerosol transmission can be mopped up using appropriate disinfectants.

5.4. Spillage inside a microbiological safety cabinet.

- These are usually well contained and can be cleaned up immediately with disinfectant. Fumigation of the cabinet will be required for CL 3 organisms and for certain CL 2 organisms [ subject to risk assessment].

5.5. Spillage in a centrifuge or orbital shaking incubator

Centrifuge

If there is reason to believe a breakage may have occurred whilst the centrifuge was running adopt the following procedure:

- Do not open lid as the bowl may contain an aerosol if the seals have failed on rotor/buckets.
- Leave for 30 minutes to allow aerosol to settle.
- Place notice on the lid to alert others as to the problem.
- Gather necessary decontamination materials.
- After 30minute, open lid carefully and spray interior with disinfectant. Exercise care if using Virkon and limit contact time to 10mins max. Rinse with a neutral pH detergent [e.g. Neutracon] and wipe with 70% alcohol.
- Inspect rotor /buckets, if intact remove and transfer to MSC for opening.
- Disinfect rotor/buckets as above whilst in the MSC.
- For CL 3 organisms or CL2 with risk of airborne transmission, respiratory protective equipment should be worn.
- If there is a possibility that the internal parts of the centrifuge have been contaminated it will be necessary to fumigate the centrifuge by suitable means. This may require the services of an outside contractor/service company. Seek advice from the Safety Office
- Orbital shakers. Before opening always check through the observation pane for signs of leaks or spills. If in doubt do not open the lid, turn off and leave at least 30 minutes before opening. For CL 3 material use table 2 to assess airborne concentrations and obtain emergency respiratory protective equipment if required. Treat as for centrifuge above.
5.6. Accidents involving sharps

- For any accident involving sharps injury or broken skin, bleeding should be encouraged and the procedure for skin contamination adopted. A First Aider should be called.
- For Group 2 & 3 BAs/GMMs, in addition to the above contact OHU for advice and seek medical attention.
- Needlestick/sharps injuries involving Group 3 Bas/GMMs will be reportable to HSE so the Safety Office must be informed immediately.
- Complete a University accident report form.

5.7. Medical Intervention.

- Where individual/individuals have been exposed and this could result in an immediate or delayed health effect, the Occupational Health Unit must be informed and the individual must seek immediate medical attention.
- Risk assessments must consider possible adverse health effects and detail what immediate action should be taken.

5.8. Post Exposure prophylaxis

- As part of the risk assessment the need and availability of post exposure prophylaxis should have been considered and discussed with the Occupational Health Unit.
- If it is available and appropriate details of any prophylactic treatment should be included in the risk assessment and in local rules for the containment facility. The need for appropriate drugs and prophylaxis to be readily available must be considered.

5.9. Reporting.

A report should be made of any accidental release/spillage, by completion of the University’s on line accident report form.

- **Group 1 organisms** - Significant spillage should be reported to the local BSO and recorded as a ‘near miss’ incident.
- **Group 2 organisms** - As above but record as ‘exposure to toxic substances or pathogenic material’. Where it is possible that this could result in an immediate or delayed risk to human health of the environment this will require a report to be made to HSE, therefore the Safety Office must be informed.
- **Group 3 organisms** - Spillage of any material outside of the MSC or other primary container, or major spillage within and MSC must be reported immediately to the local BSO and the Safety Office and on the online reporting system. A report will be made to HSE.
- **Needlestick/sharps injuries** involving Group 3 BAs/GMMs are reportable to HSE so the Safety Office must be informed immediately.
6. Storage requirements
COSHH regulations require the safe storage of BAs. In addition storage of GMMs is a ‘contained use’ activity and as such is subject to the requirements of the Contained Use Regulations namely:

- Risk assessment
- Application of appropriate control measures
- Classification of the activity
- Notification where appropriate.

Table 4 gives details of storage requirements for BAs/GMMs.

6.1. Archive material
Wherever possible material associated with experiments that are no longer 'active' and are not covered by a current risk assessment should be disposed of. Where archive material has to be kept and is not covered by a current assessment it must be physically segregated from currently used material. The outer container must be labelled to the effect that the material inside must not be used unless a risk assessment is carried out and submitted to the GMSC for approval.
<table>
<thead>
<tr>
<th>Table 4</th>
<th><strong>Level 1</strong></th>
<th><strong>Level 2</strong></th>
<th><strong>Level 3</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General Requirements</strong></td>
<td>A nominated individual must maintain an inventory of all organisms stored within the laboratory. It is important that every individual vial/plate can be traced back to a risk assessment.</td>
<td>As for level 1</td>
<td>Storage should be within the level 3 containment facility. Where this is not practicable it may be permissible to use a fridge or freezer in an adjacent area providing that &lt;ul&gt;&lt;li&gt;It is directly adjacent to the CL 3 facility.&lt;/li&gt;&lt;li&gt;It is kept locked and secure at all times – with key access controlled by a responsible named person.&lt;/li&gt;&lt;/ul&gt; Transport to the storage location should be by secure means and should not necessitate going out onto a corridor.</td>
</tr>
<tr>
<td><strong>Labelling requirements for storage vials and growth vessels.</strong></td>
<td>All viable organisms, irrespective of where they are stored/used must be clearly labelled with &lt;ul&gt;&lt;li&gt;parental organism,&lt;/li&gt;&lt;li&gt;vector&lt;/li&gt;&lt;li&gt;insert&lt;/li&gt;&lt;li&gt;date&lt;/li&gt;&lt;li&gt;initials of owner.&lt;/li&gt;&lt;/ul&gt;</td>
<td>As for level 1</td>
<td>As for level 1</td>
</tr>
<tr>
<td><strong>Room temperature storage</strong></td>
<td>Due to the nature of the work such storage is likely to be relatively short term. &lt;br&gt;&lt;br&gt;Agar plates, universals and other vessels containing liquid cultures must be stored securely in such a way as they are not in danger of being spilt or knocked over. This can be achieved by: &lt;ul&gt;&lt;li&gt;Storing in trays&lt;/li&gt;&lt;li&gt;Use of suitable tube racks&lt;/li&gt;&lt;li&gt;Ensuring cultures /plates are not near the edge of benches or in areas where there is danger of spillage.&lt;/li&gt;&lt;/ul&gt;</td>
<td>As for level 1, where risk assessment has identified that short term storage on bench is acceptable.</td>
<td>Viable material must not be left outside of the Safety Cabinet when not in use.</td>
</tr>
<tr>
<td><strong>Level 1</strong></td>
<td><strong>Level 2</strong></td>
<td><strong>Level 3</strong></td>
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<tr>
<td><strong>Fridge/4°C</strong></td>
<td><strong>Agar plates</strong> must be sealed with para-film and secured in stacks. <strong>Liquid cultures</strong> must be secured in racks or placed in suitable outer [secondary] containers to reduce risk of spillage. Segregate viable organisms from other non viable materials in fridge. The fridge must bear a <strong>biohazard</strong> sign.</td>
<td>As for level 1 - plus Fridge dedicated solely for GM storage Located within a designated Level 2 areas.</td>
<td>As for level 1 &amp; 2 – fridge must be within the CL 3 room.</td>
</tr>
<tr>
<td><strong>-20°C /80°C</strong></td>
<td>BAs/GMMs are usually contained in small ampoules of 2ml or less. These should be secured in trays within boxes. The box must be appropriately labelled as to its contents and bear a biohazard sign. It is recognised that in many cases it will not be practicable to dedicate an entire freezer for this purpose. In these circumstances a shelf or area of the freezer should be designated for storage of BAs/GMMs and should bear biohazard signs. The box must be sealed and leak-proof to contain liquid in the event of freezer failure. It may also be necessary to store some frozen stocks in -80°C freezers which may be remote from the designated GM laboratory. The door of the freezer must also have a biohazard sign and the freezer must be kept locked if outside of the facility.</td>
<td>As for level 1 – Where the freezer is not within the GM laboratory it must be kept locked. On no account must freezers containing level 2 GMMs be located on corridors. Transport to the freezer should be by secure means to minimise risk of spillage.</td>
<td>Freezers should be within the level 3 containment facility. Where this is not reasonably practicable it may be permissible to use a fridge or freezer in an adjacent area providing that • It is directly adjacent to the CL 3 facility. • It is kept locked and secure at all times – with key access controlled by a responsible named person. • Transport to the storage location should be by secure means and should not necessitate going out onto a corridor.</td>
</tr>
</tbody>
</table>
7. Transport of BAs/GMMs

COSHH regulations require the safe transport of BAs. In addition transport of GMMs is a 'contained use' activity and as such is subject to the requirements of the Contained Use Regulations namely:

- Risk assessment
- Application of appropriate control measures
- Classification of the activity
- Notification where appropriate.

7.1. Transport between and within laboratory areas in the same building.

Facilities should be so designed to remove the need to transport viable BAs/GMMs any distance. It is particularly important to avoid the need to carry them through communal areas or circulation routes. Where this cannot be avoided transport must be such as to avoid or reduce the risk of spillage.

**NOTE:** Liquid cultures of Group 3 BAs/GMMs must not leave the containment facility.

For low risk organisms the following must be observed:

- Particular care should be taken when liquid cultures are being transported within the lab or between labs.
- Use trays and tube racks to carry cultures around the laboratory.
- Small volumes of culture that can easily be carried must be transported within a secondary outer container with a lid. If doors need to be negotiated use a trolley or get assistance from a colleague.
- Larger cultures must be moved on a trolley, in a secure manner using additional trays and tube racks as required.
- On no account must glass flasks containing viable organisms be carried without the use of secondary containment.
- Individuals transporting cultures outside of the laboratory should ensure they have enough absorbent material and gloves readily available to deal with any spillage.

7.2. Transport outside.

A separate guidance document [Code of Practice for the Transport of Dangerous Goods] is available which gives full details of the requirements for transport of BAs/GMMs to other sites within the University or to other organisations or institutions. This can be found on Safety Office web site.
8. References & sources of information

The Code of Practice was drawn up with reference to the following documents:


2. SACGM Compendium of Guidance

3. Genetically Modified Organisms (Contained Use) Regulations 2003


5. ACDP Approved list of biological agents, 2004
